Journal of Current Hematology&Oncology Research

Original Article

DOI: 10.51271/JCHOR-0035

Does early granulocyte colony-stimulating factor administration in autologous peripheral blood stem cell transplantation shorten the duration of hospitalization in patients with multiple myeloma?

Muzaffer Keklik[1], Leyla Topal[2], Serhat Çelik[3], Leylagül Kaynar[4]

[1]Department of Hematology, Erciyes University Faculty of Medicine, Kayseri, Turkiye
[2]Department of Internal Medicine, Goksun State Hospital, Kahramanmaraş, Turkiye
[3]Department of Hematology, Yenimahalle Training and Research Hospital, Yıldırım Beyazıt University, Ankara, Turkiye
[4]Department of Hematology, Istanbul Medipol University Faculty of Medicine, İstanbul, Turkiye

Cite this article: Keklik M, Topal L, Çelik S, Kaynar L. Does early granulocyte colony-stimulating factor administration in autologous peripheral blood stem cell transplantation shorten the duration of hospitalization in patients with multiple myeloma?.*J Curr Hematol Oncol Res.* 2024;2(2):41-46.

Corresponding Author: Muzaffer Keklik, muzafferkeklik@gmail.com

Received: 21/02/2024 ◆ **Accepted**: 03/04/2024 ◆ **Published**: 14/05/2024

ABSTRACT

Aims: Autologous peripheral blood stem cell transplantation (PBSCT), performed with high-dose melphalan support following induction therapy is still the gold standard method of treatment for multipl myeloma (MM)patients suitable for transplantation. It was aimed, with this retrospective study, to investigate the effects of early (1 day after PBSCT) and late (5 days after PBSCT) initiation of granulocyte colony-stimulating factor (G-CSF) support following PBSCT on engraftment time, febrile neutropenia, and length of hospital stay (LOS) in MM patients.

Methods: This study included 70 patients with MM, who underwent PBSCT in Erciyes University. Two groups were administered 5µg/kg filgrastim, subcutaneously, either 1 day or 5 days after PBSCT, until neutrophil engraftment was reached.

Results: Both neutrophil and platelet engraftment occurred in significantly shorter times in the early G-CSF group compared to late G-CSF group; the median times to neutrophil engraftment were 10 (8-13) and 11 (7-15) days, respectively, and the median times to platelet engraftment were 11 (10- 16) and 13 (11- 21) days (p=0.001). Also, the median LOS was also significantly shorter in the early G-CSF group compared to late G-CSF group; 14 (10-22) vs 16 (11- 33) days, respectively (p=0.016). No significant difference was found between the groups in terms of frequency of febrile neutropenia.

Conclusion: The initiation of G-CSF support early, following PBSCT in MM patients, accelerated neutrophil and platelet engraftment and shortened the LOS as compared to the initiation of G-CSF support late, with no significant difference in the frequency of febrile neutropenia.

Keywords: Autologous hematopoietic stem cell transplantation, granulocyte colony-stimulating factor, multiple myeloma

INTRODUCTION

Autologous peripheral blood stem cell transplantation (PBSCT), is still the standard method of treatment following high-dose chemotherapy in multiple myeloma (MM) patients eligible for transplant, yet the complications associated with prolonged neutropenia have led transplant centers to seek other treatment methods.[1] Administration of granulocyte colony-stimulating factor (G-CSF) after conditioning chemotherapy and stem cell infusion has been shown to expedite neutrophil recovery, decrease time to neutrophil engraftment, and decrease the risk of febrile neutropenia.[2-4] In parallel, the American Society of Clinical Oncology (ASCO) and The National Comprehensive Cancer Network (NCCN) guidelines recommend that G-CSF support should be initiated 1 to 5 days after the administration of high-dose chemotherapy and continued until an absolute neutrophil count (ANC) of 2-3×10^9/L is reached.[5,6] Nevertheless, the data on the optimal timing to initiate G-CSF support is limited and also contradictory.[2,7-9] Also, there are only a few studies that investigated the effect of the timing of the administration of the G-CSF support after PBSCT specifically in the context of MM patients. In this study, the effects of initiating G-CSF early (day 1) or late (day 5) after PBSCT on the neutrophil and platelet engraftment, development of febrile neutropenia, and duration of hospitalization were compared in MM patients. Additionally, the effect of pre-transplant radiotherapy (RT) history on engraftment times was investigated.

This work is licensed under a Creative Commons Attribution 4.0 International License.

METHODS

The study was carried out with the permission of Erciyes University Faculty of Medicine Medical Ethics Committee (Date: 03.11.2020, Decision No: 2020/563). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This retrospective cohort study conducted at Transplantation Center of Erciyes University included adult (age ≥18 years) MM patients undergoing PBSCT using high-dose melphalan conditioning between November 2015, and July 2020. Demographic and clinical features of the patients, induction treatments administered, and their remission statuses before transplantation and history of pre-transplant RT were recorded. Their responses to the treatment before PBSCT were evaluated according to International Myeloma Working Group (IMWG) criteria.[10]

Cyclophosphamide (CY) and G-CSF was used as the mobilization regimen in 31 patients in the first group and 29 patients in the second group. Accordingly, 2.4 g/m^2 CY was administered to the patients on day +1 via intravenous (IV) infusion for two hours, accompanied by Mesna (2-mercaptoethane sulfonate Na) and adequate hydration for the prevention of haemorrhagic cystitis. As the G-CSF, filgrastim (Neupogen, Amgen) 10 µg/kg/d dose was divided into two and started subcutaneously to be administered on day +5 and continued to be administered until sufficient amount of CD34+ cells were collected. Prior to chemotherapy, granisetron or ondansetron, pheniramine maleate, and dexamethasone were administered as IV infusion, whereas acetazolamide support was administered orally. Also this group was given levofloxacin, acyclovir and fluconazole prophylactically, G-CSF was administered along with plerixafor (Mozobil, Genzyme Corp) support due to lack of mobilization in four patients in the first group and five patients in the second group. These patients were administered the filgrastim 10 µg/kg/d dose was divided into two and started subcutaneously to be administered for at least five days and 0.24 mg/kg plerixafor subcutaneously on the fourth day, as suggested in the literature.[11] Additionally, one patient in the second group was mobilized with G-CSF alone; filgrastim 10 µg/kg/d dose was divided into two and started subcutaneously to be administered on day +1 and continued to be administered until sufficient amount of CD34+ cells were collected. The peripheral complete blood count (CBC) measurement was started on day +8 and continued to be performed every other day. Peripheral blood CD34+ cell count was measured daily when patient's white blood cell count recovered to ≥4.000/µL. When CD34+ cell count was ≥10/µL, apheresis was started. Consequentially, adequate doses of CD34+ cells were collected in all patients using a Spectra Optia Apheresis System (Terumo BCT, Lakewood, Colorado, U.S.). Measurements of peripheral blood CD34+ cell count and CD34+ cell content of the apheresis product were performed by the BD FACSCalibur flow cytometer (Becton–Dickinson, Erembodegem, Belgium). The harvested cells were cryopreserved in 10% dimethyl sulfoxide (DMSO) using a controlled-rate freezer, and then stored in liquid nitrogen.

Approximately 2–3 weeks after the mobilization, all patients received conditioning with 200 mg/m² melphalan (140 mg/m^2 in patients with renal insufficiency or >65 years old) two days before the infusion of autologous stem cells, followed by autologous PBSCT on day 0. While the patients who underwent PBSCT between November 2015 and May 2018 received G-CSF at a dose of 5 µg/kg/day subcutaneously starting on post-transplantation on day +5, the patients who underwent PBSCT between May 2018 and July 2020 received the G-CSF at the same dose starting on post-transplantation on day +1. The patients in both groups continued G-CSF treatment until neutrophil engraftment was reached. Also the patients in both groups were given anti-infective prophylaxis, which included 500 mg levofloxacin taken daily, 500 mg valacyclovir taken twice daily, and 400 mg fluconazole taken daily, in accordance with institutional policy. Post-PBSCT neutrophil and platelet engraftment time, development of neutropenic fever, and duration of hospitalization of the two groups were recorded. Neutrophil engraftment was considered the first of three successive days with an ANC≥0.5 x 10^9/L. Also platelet engraftment was considered the first of three consecutive days with a platelet count ≥20x10^9/L. Additionaly, febrile neutropenia was considered the present the fever was ≥38°C and ANC was <0.5×10^9/L from the day of PBSCT until the day of neutrophil engraftment.

Statistical Analysis

SPSS 22.0 (IBM Statistical Package for Social Sciences for Windows, version 22.0, IBM Corp., Armonk, NY, U.S.) software package was used for statistical analyses. The Kolmogorov-Smirnov test was used to check whether the research data conformed to normal distribution or not. Pearson's chi-squared test was used to analyze the independent qualitative data, whereas the student's t-test was used to analyze the independent quantitative data. The Mann-Whitney U test was used to analyze non-normally distributed parameters. Probability (p) values of <0.05 were deemed to indicate statistical significance.

RESULTS

The baseline characteristics of the patients are presented in Table 1. There were 35 patients in each group. The groups were well balanced in terms of age, sex, paraprotein types, disease stage, induction therapies administered, and pre-transplant disease status. Also the groups did not differ significantly in terms of pre-transplant RT history, mobilization protocol, conditioning regimen and CD34+ cell dose.

No serious side effects were observed in both groups during the mobilization and transplantation process. The median time to neutrophil engraftment was 10 days (interquartile range [IQR], 8-13 days) in the early G-CSF group compared with 11 days (IQR, 10-16 days) in the late G-CSF group (p< 0.001) (Table 2 and Figure 1). Also, the median time to platelet engraftment was 11 days (IQR, 7-15 days) in the early G-CSF group compared with 13 days (IQR, 11-21 days) in the late group (p< 0.001) (Table 2 and Figure 2). Additionally, the duration of post-PBSCT hospitalization was 13 days (IQR, 10-22 days) in

the early G-CSF group compared to 16 days (IQR, 11-25 days) in the late group (p= 0.02) (Table 2 and Figure 3).

Table 1. Patient and transplant characteristics

Variables	Early (n=35)	Late (n=35)	P value
Age,yr, median (IQR)	56 (44-64)	57 (40-65)	0.41
Sex, n (%)			0.32
Male	20 (57.1)	24 (68.6)	
Female	15 (42.9)	11 (31.4)	
Isotype, n (%)			0.91
Ig G	25 (71.4)	23 (65.7)	
Ig A	5 (14.2)	8 (22.8)	
Ig D	1 (2.9)	1 (2.9)	
Nonsecretuar	1 (2.9)	1 (2.9)	
Other	3 (8.6)	2 (5.7)	
R-ISS, n (%)			0.26
I	24 (68.6)	27 (77.1)	
II	5 (14.3)	4 (11.4)	
III	1 (2.8)	1 (2.9)	
Missing	5 (14.3)	3 (8.6)	
Pretransplant therapy, n (%)			0.11
VAD+VCD	19 (54.3)	16 (45.7)	
VCD	13 (37.1)	16 (45.7)	
VCD+RD	3 (8.6)	3 (8.6)	
Pretransplant radiotherapy, n (%)			0.16
Yes	11 (31.4)	6 (17.1)	
No	24 (68.6)	29 (82.9)	
Pretransplantation status, n (%)			0.87
CR+VGPR	22 (62.9)	26 (54.5)	
PR	13 (37.1)	9 (45.5)	
Mobilization, n (%)			0.37
Cyclophosphamide + G-CSF	31 (88.6)	29 (82.8)	
Cyclophosphamide + G-CSF+ plerixafor	4 (11.4)	5 (14.3)	
G-CSF only	0	1 (2.9)	
Conditioning regimen,n (%)			0.55
Melphalan 140 mg/m^2	8 (22.9)	6 (17.1)	
Melphalan 200 mg/m^2	27 (77.1)	29 (82.9)	
CD34+ dose, x 10^6 cells/kg, median (IQR)	4.95 (3.22-10.31)	4.99 (3.30-9.86)	0.63

IQR: Interquartile range, R-ISS: Revised international staging system, VAD: Vincristine adriamycin dexamethasone, VCD: Bortezomib cyclophosphamide dexamethasone, RD: Lenalidomide dexamethasone, CR: Complete recovery, VGPR: Very good partial recovery, PR: Partial recovery, G-CSF: Granulocyte colony-stimulating factor

These results were statistically significant. There was no significant difference between the groups regarding the platelet or erytrocyte transfusion requirement. Febrile neutropenia occurred in 20 patients (57.1%) in the early group and 18 patients (51.4%) in the late group (p=0.81). Those patients were treated with infusion of antimicrobial agents and their febrile neutropenia was resolved successfully. There was no relevant difference in both groups in terms of the frequency of febrile neutropenia, relationship of pre-transplant RT history and engraftmen time.

Table 2. Clinical outcomes

	Early (n=35)	Late (n=35)	P value*
Time to neutrophil engraftment,d, median (IQR)	10 (8-13)	11 (10-16)	<0.001
Time to platelet engraftment,d, median (IQR)	11 (7-15)	13 (11-21)	<0.001
Febrile neutropenia,n (%)	20 (57.1)	18 (51.4)	0.81
Duration of hospitalization post-PBSCT,d, median (IQR)	13 (10-22)	16 (11-25)	0.02

IQR: Interquartile range, PBSCT: Peripheral blood stem cell transplantation, *: Statistically significant

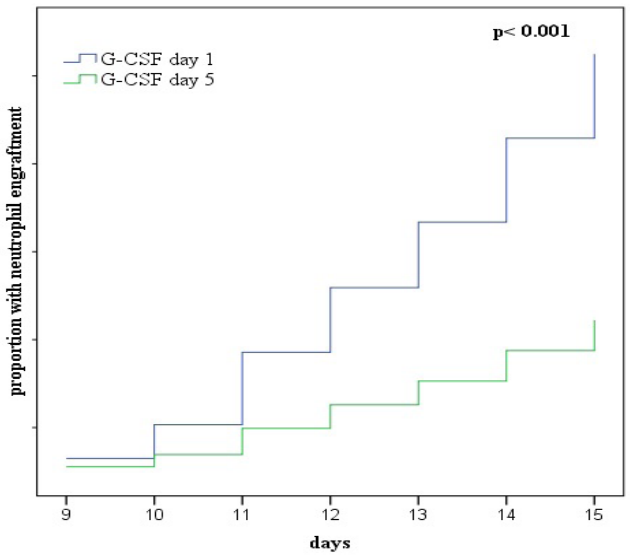

Figure 1. Time to neutrophil engraftment

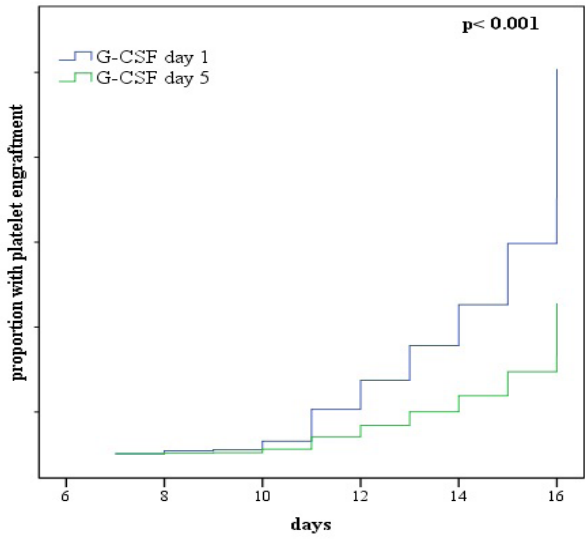

Figure 2. Time to platelet engraftment

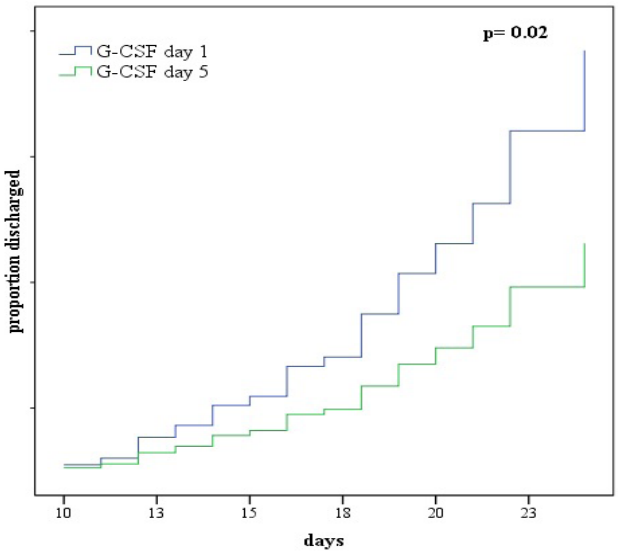

Figure 3. Duration of hospitalization post-transplantation

DISCUSSION

The findings of this study revealed that initiation of G-CSF support early following PBSCT in MM patients accelerated neutrophil and platelet engraftment and shortened the duration of hospitalization. There is no consensus in the literature on the optimum timing to initiate G-CSF support in the post-transplant period. To cite a few examples, in a study by Thompson et al.[7] initiation of G-CSF support on the same day after PBSCT was compared to initiation of G-CSF support five days after PBSCT in the context of various hematological diseases. Consequently, the median time to neutrophil engraftment in the group of patients, who received G-CSF support early, was found as 10 (7-27) days, as compared to 11 (9-15) days in the group of patients, who received G-CSF support late, which indicated a significant difference between the groups in favor of the patient group, who received G-CSF support early (p<0.001). Additionally, in the same study, no significant difference was found between the groups in terms of platelet engraftment times. In a study by Valteau-Couanet et al.[12], patients, who were started on G-CSF support one day after PBSCT, patients who were started on G-CSF support five days after PBSCT, and patients who did not receive G-CSF support, were compared in the context of various hematologic and oncologic malignancies. Consequently, it was determined that the neutrophil engraftment times in the patient groups that received G-CSF support were significantly shorter than those of the patient group that did not receive G-CSF support, whereas there was no difference between the groups in terms of platelet engraftment times. Additionally, in the same study, the neutrophil engraftment times in the patient group that was started on G-CSF support one day after PBSCT, the patient group that was started on G-CSF support five days after PBSCT, and the patient group that did not receive G-CSF support, were found as 9 (4- 40), 10 (5-15), and 13 (7-36) days, respectively. Thus, indicating a significant difference in favor of the patient groups that received G-CSF support (p<0.0001). Furthermore, duration of hospitalization was found to be significantly shorter in the patient groups that received G-CSF support, as compared to the patient group that did not receive G-CSF support. In another study, the difference between the administration of G-CSF support five days after PBSCT empirically and 12 days after PBSCT on patients with an ANC count of <0.5x10^9/L was investigated in terms of engraftment times and duration of hospitalization in patients with MM and lymphoma.[13] Consequently, the neutrophil engraftment times in the patient group that was administered G-CSF support five days after PBSCT and in the patient group that was administered G-CSF support 12 days after PBSCT were found as 12 days and 13 days, respectively. This indicated a significant difference in favor of the patient group that received G-CSF support early (p=0.07). Additionally, in the same study, febrile neutropenia incidences in the patient group that was administered G-CSF support five days after PBSCT and in the patient group that was administered G-CSF support 12 days after PBSCT were reported as 74% and 90%, respectively. This also indicated a significant difference in favor of the patient group that received G-CSF support early (p=0.04). However, no significant difference was found between the groups in terms of platelet engraftment time and duration of hospitalization. The patient group in all these studies consisted of various diseases such as MM and/or lymphoma, solid tumor. In our study, only the data belonging to the MM patients were presented. Few studies were investigated the effect of the timing of the administration of G-CSF support after PBSCT, specifically in the context of MM patients. In one of these studies, Sborov et al.[8] compared the initiation of G-CSF support in MM patients one day, five days, and seven days after PBSCT, and found that the neutrophil engraftment time was shorter and the incidence of neutropenic fever was lower in the group of patients that received G-CSF earlier than others. In the same study, the neutrophil engraftment times in the patient groups that were started on G-CSF support one day, five days, and seven days after PBSCT were found as 12.8 days, 12.3 days, and 11.2 days, respectively. This indicated a significant increase in the patient group that was started on G-CSF support seven days after PBSCT, as compared to the other groups (p<0.001). Additionally, the duration of severe neutropenia was found to be significantly increased in the patient groups that were started on G-CSF support five days and seven days after PBSCT, as compared to the patient group that was started on the G-CSF support one day after PBSCT. Besides that there are two patient groups in our study when there are three patient groups in this study, neutrophil engraftment was occurred in shorter periods in both groups in our study. In addition, a severe neutropenia increase in both groups was mentioned in this study while no such result was found in our study.

In another study, Jackson et al.[14] compared the MM patient group that was administered G-CSF support after PBSCT with the MM patient group, which was not administered G-CSF support after PBSCT, and reported that both engraftment times and duration of hospitalization were significantly less in the patient group that was administered G-CSF support after PBSCT. Additionally, the median times to neutrophil engraftment and the median duration of hospitalization in the said patient groups were found as 12 days and 19 days, and as 15 days and 17 days, respectively, indicating a significant difference between the groups in both categories in favor of the patient group that was administered G-CSF support after PBSCT (p<0.001 and p=0.026). The difference of this study from our study is that no G-CSF support was given to one of the groups, while the other group was given G-CSF in the late period (4-20 days). Also, although the duration of hospitalization with neutrophil engraftment in the group

given G-CSF support was significantly short compared to the group that was not given at all, it was long compared to our early G-CSF group. This result also supports our idea that early G-CSF application is advantageous.

Yet in another study, Cox et al.[15] reported the median neutrophil engraftment times and duration of hospitalization in patient groups that were started on G-CSF support seven days and 14 days after PBSCT as 12 days and 15 days and as 17 days and 19 days, respectively, indicating significant differences between the groups in both categories in favor of the patient group that was administered G-CSF support early ($p<0.0001$ for both cases). G-CSF application days were also different in this study from our study. Moreover, the relationship between the history of pre-transplant RT and engraftment kinetics was not examined in any of these studies. As is seen, there is no research in the literature that our study exactly overlaps.

As for the effect of having a history of pre-transplant RT on the engraftment times, no significant effect was observed in either patient group. This result differs from the relevant results reported in the literature, which indicated that pre-transplant RT significantly affected and delayed platelet engraftment. In those studies, those results may be associated with distorted marrow microenvironment due to RT.[16,17] Data belonging to various cancer patients were shared in those studies.

Limitations

There are some limitations to our study. The first is that patients were not evaluated in terms of total survival and relapse. Because most of the patients who are transplanted in our center come from other provinces and can have their follow-up done in the provinces where they are located after the transplantation. The second might be the cost analysis. The costs of all procedures in the transplantation process of the groups, mobilization and transplant preparation regimes can be affected by various factors. For example, depending on the condition of the patient and the clinic, the mobilization process may be performed by hospitalization or outpatient follow-up. The use of plerixafor in case of failure in mobilization affects the cost. In other words, it may be appropriate to plan a prospective study for a cost analysis based on the duration of post-transplantation hospitalization.

CONCLUSION

It was found that initiating the G-CSF support one day after PBSCT, as compared to five days after PBSCT, significantly shortened the neutrophil and platelet engraftment times as well as duration of hospitalization in MM patients. The results of this study support the transplant centers that reported a positive contribution of early G-CSF support on engraftment and duration of hospitalization.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Erciyes University Faculty of Medicine Medical Ethics Committee (Date: 03.11.2020, Decision No: 2020/563).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. *N Engl J Med*. 1996;335(2):91-97. doi: 10.1056/nejm199607113350204
2. Trivedi M, Martinez S, Corringham S, Medley K, Ball ED. Optimal use of G-CSF administration after hematopoietic SCT. *Bone Marrow Transplant*. 2009;43(12):895-908.
3. Klumpp TR, Mangan KF, Goldberg SL, Pearlman ES, Macdonald JS. Granulocyte colony-stimulating factor accelerates neutrophil engraftment following peripheral-blood stem-cell transplantation: a prospective, randomized trial. *J Clin Oncol*. 1995;13(6):1323-1327.
4. Schmitz N, Ljungman P, Cordonnier C, et al. Lenograstim after autologous peripheral blood progenitor cell transplantation: results of a double-blind, randomized trial. 2004;34(11):955-962.
5. Smith TJ, Bohlke K, Lyman GH, et al. Recommendations for the use of WBC growth factors: American Society of Clinical Oncology clinical practice guideline update. *J Clin Oncol*. 2015;33(28):3199-3212.
6. Becker PS, Griffiths EA, Alwan LM, et al. NCCN guidelines insights: hematopoietic growth factors, version 1.2020: featured updates to the NCCN guidelines. *J National Comprehens Canc Netw*. 2020;18(1):12-22.
7. Thompson JM, Carlton P, Akard LP, Dugan MJ, Jansen J. Starting granulocyte-colony-stimulating factor (filgrastim) early after autologous peripheral blood progenitor cell transplantation leads to faster engraftment without increased resource utilization. *Transfusion* 2009;49(3):548-554. doi: 10.1111/j.1537-2995.2008.02006.x
8. Sborov DW, Cho YK, Cottini F, et al. G-CSF improves safety when you start the day after autologous transplant in multiple myeloma. *Leukem Lymph*. 2017;58(12):2947-2951. doi: 10.1080/10428194.2017.1318436
9. Singh AD, Parmar S, Patel K, et al. Granulocyte colony-stimulating factor use after autologous peripheral blood stem cell transplantation: comparison of two practices. *Biol Blood Marrow Transplant*. 2018;24(2):288-293.

10. Hillengass J, Usmani S, Rajkumar SV, et al. International myeloma working group consensus recommendations on imaging in monoclonal plasma cell disorders. *Lancet Oncol*. 2019;20(6):e302-e312. doi: 10.1016/S1470-2045(19)30309-2
11. Tekgündüz E, Altuntaş F, Şıvgın S, et al. Plerixafor use in patients with previous mobilization failure: a multicenter experience. *Transfus Apheres Sci*. 2012;47(1):77-80. doi: 10.1016/j.transci.2012.05.004
12. Valteau-Couanet D, Faucher C, Auperin A, et al.Cost effectiveness of day 5 G-CSF (Lenograstim®) administration after PBSC transplantation: results of a SFGM-TC randomised trial. *Bone Marrow Transplant*. 2005;36(6):547-552.
13. Singh AD, Parmar S, Patel K, et al. Granulocyte colony-stimulating factor use after autologous peripheral blood stem cell transplantation: comparison of two practices. *Biol Blood Marrow Transplant*. 2018;24(2):288-293.
14. Jackson ER, Jared JR, Piccolo JK, et al. Granulocyte colony-stimulating factor utilization postautologous hematopoietic stem cell transplant in multiple myeloma patients: does one size fit all? *J Oncol Pharm Pract*. 2019;2(5):1135-1141.
15. Cox J, Campos S, Wu J, et al. Efficacy of deferred dosing of granulocyte colony-stimulating factor in autologous hematopoietic transplantation for multiple myeloma. *Bone Marrow Transplant*. 2014;49(2):219-222.
16. Gonçalves TL, Bennvegnu DM, Bonfanti G. Specific factors influence the success of autologous and allogeneic hematopoietic stem cell transplantation. *Oxidat Med Cellul Longev*. 2009;2(2):82-87. doi: 10.4161/oxim.2.2.8355
17. Turk HM, Komurcu S, Arpaci F, et al. Factors affecting engraftment time in autologous peripheral stem cell transplantation. *Asian Pacif J Cancer Prevent*. 2010;11(3):697-702.